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Decrease in CuZn-superoxide dismutase mRNA level during differentiation of human monocytic and promyelotic leukemia cells

Hiroyuki Saito, Toshio Kuroki and Kiyoshi Nose

Department of Cancer Cell Research, The Institute of Medical Science, The University of Tokyo, Shirokanedai, Minatoku, Tokyo 108, Japan

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Change in the level of CuZn-superoxide dismutase (SOD) mRNA was examined using a molecular probe during differentiation of human monocytic leukemia U937 cells or promyelotic leukemia HL-60 cells induced by either 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) or dimethylsulfoxide (DMSO). CuZn-SOD mRNA levels were found to decrease during the course of differentiation, and this response is specific for differentiation, since the treatment of human B cell leukemia cells or normal diploid fibroblasts with TPA failed to have any effect on the level of CuZn-SOD mRNA. The activity of CuZn-SOD in U937 cells also decreased during differentiation, but following that of the CuZn-SOD mRNA level. The expression of the CuZn-SOD gene is thus concluded to diminish during the differentiation of HL-60 and U937 cells.

Superoxide dismutase; Differentiation; Oxygen, active; (U937 cell, HL-60 cell)

1. INTRODUCTION

Human monocytic leukemia U937 promyelotic leukemia HL-60 cells are known to terminally differentiate into monocyte-like cells in the presence of TPA and HL-60 cells in DMSO to differentiate into granulocyte-like cells [1,2]. Differentiated cells have various macrophage- or granulocyte-specific functions, such as phagocytosis, and Fc-receptors of non-specific esterases (for review [3]). The expression of the proto-oncogene c-*myc* is diminished during the differentiation of HL-60 or U937 cells [4–7], whereas that of c-*sis* and c-*fms* genes increases during TPA-induced differentiation [8]. Besides these phenotypic changes, differentiated HL-60 and U937 cells produce superoxide anions [9].

The active oxygens which include superoxide anions are extremely toxic and clastogenic to cells [10], but we have recently observed that active oxygens can work as growth stimuli at appropriate concentrations [11,12]. Similar findings on the 'positive' effect of active oxygens have been reported by Cerutti and his colleagues [13,14]. Active oxygens are also supposed to play a role in cell differentiation [15].

Superoxide dismutase (SOD) is thought to be one of the key enzymes in controlling superoxide anion and other active oxygen levels [16], and some authors considered it to be involved in tissue development [17]. It was thus considered pertinent to examine the expression of SOD during the differentiation of HL-60 or U937 cells. The present communication reports that during differentiation, there is a decrease in the level of CuZn-SOD mRNA and its activity.

Correspondence address: K. Nose, Department of Cancer Cell Research, Institute of the Medical Science, University of Tokyo, Shirokanedai, Minatoku, Tokyo 108, Japan

Abbreviations: DMSO, dimethylsulfoxide; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate

2. MATERIALS AND METHODS

2.1. *Cells and chemicals*

U937, HL-60 and Raji cells were obtained from the Japanese Cancer Research Resources Bank and grown in RPMI-1640 supplemented with 10% fetal bovine serum in 95% air and 5%

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CO_2. Normal human fibroblast JHU-1 cells were cultured as described previously [18]. Cells were plated at approx. 1×10^5 cells/ml in 100 mm plastic dishes and treated with inducers 3 days after plating.

TPA and 1-oleoyl-2-acetylglycerol (OAG) were obtained from Sigma Chemicals and Serdary Research Lab., respectively.

2.2. *Analysis of RNA*

Total cellular RNA was extracted by the guanidinium-hot phenol method as described previously [19] and run on a 1.5% agarose gel containing 2.2 M formaldehyde. After transfer of the RNA to nylon membranes (Hybond N, Amersham), the filters were hybridized with ^{32}P-labeled molecular probes. Oligonucleotides for human CuZn-SOD cDNA corresponding to nucleotides 93–142 (50-mer) [20] and human extracellular SOD cDNA corresponding to nucleotides 70–119 (50-mer) [21] were synthesized and labeled with random primers and Klenow enzyme (Pharmacia). c-*myc* [18] and β-actin probes [19] were as described previously.

2.3. *Measurement of CuZn-SOD activity*

The cells were washed with phosphate-buffered saline and disrupted by sonication in 0.1 M KH_2PO_4, pH 8.2. CuZn-SOD activity was measured by the nitrite-kit method following the procedure of Oyanagi [22] which uses xanthine/xanthine oxidase to produce O_2^- and hydroxyamine/*N*-1-naphthylethylenediamine dihydrochloride + sulfanilic acid to produce color. The activity, assayed in the presence of 5 mM KCN (Mn-SOD), was subtracted from total SOD activity and the value thus obtained was regarded to be the activity of CuZn-SOD.

3. RESULTS AND DISCUSSION

The level of CuZn-SOD mRNA was determined using Northern blot hybridization of total RNA extracted from monocytic leukemia U937 cells induced to differentiate by TPA. RNA was fractionated on a 1.5% agarose gel containing formaldehyde and hybridized with a synthetic oligonucleotide probe specific for CuZn-SOD cDNA. The probe detected a single band of approximately 0.7 kb in size (fig.1). The amount of CuZn-SOD mRNA was found to decrease during the course of differentiation of U937 cells treated with 50 ng/ml of TPA, whereas the amount of β-actin mRNA used as a reference remained essentially unchanged or increased only slightly. U937 cells treated with TPA for 24 h were clearly shown to differentiate by morphological changes and the appearance of non-specific esterases and the Fc-receptor. Fractions of Fc-receptor- and non-specific esterase-positive cells were 11% and 8.6%, respectively, in undifferentiated cells and were increased to 31% and 42% after treatment with 50 ng/ml TPA for 24 h.

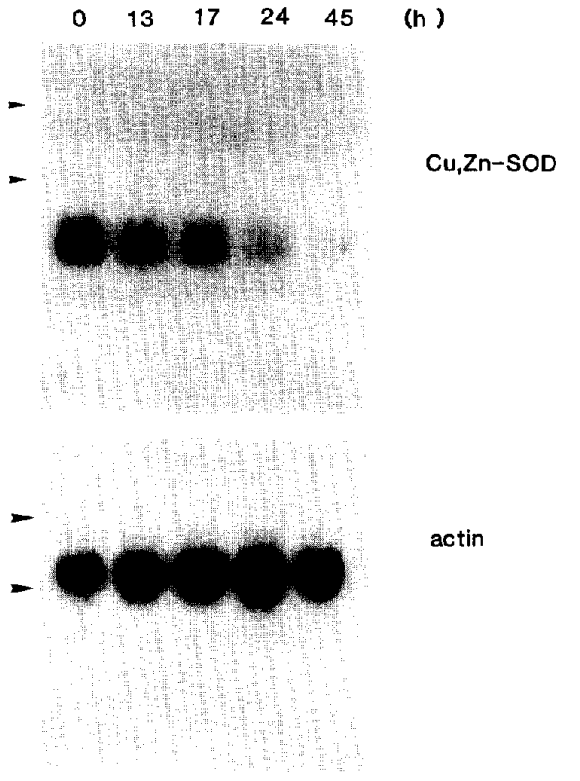

Fig.1. Changes in the level of CuZn-SOD mRNA during differentiation of U937 cells. Cells were incubated with 50 ng/ml TPA for the times indicated. Total RNA (20 μg/lane) was extracted and run on an 1.5% agarose gel containing 2.2 M formaldehyde. After being transferred to nylon membranes, the filters were hybridized with ^{32}P-labeled human CuZn-SOD or β-actin probes. The arrows indicate the positions of 28 and 18 S rRNA.

Protein kinase C is activated by TPA as well as by synthetic diglycerol, OAG [23], and this activation is thought to be essential for differentiation. Kreuter et al. [24], however, reported that HL-60 cells were induced to differentiate by TPA but not by OAG. To determine whether the decrease in CuZn-SOD mRNA level is correlated with differentiation, U937 cells were treated with either 50 ng/ml TPA or 50 μg/ml OAG. TPA treatment caused a dramatic decrease in the c-*myc* mRNA level which is known to be associated with differentiation of leukemia cells, whereas OAG treatment failed to cause such a change (fig.2). CuZn-SOD mRNA levels in OAG-treated cells did

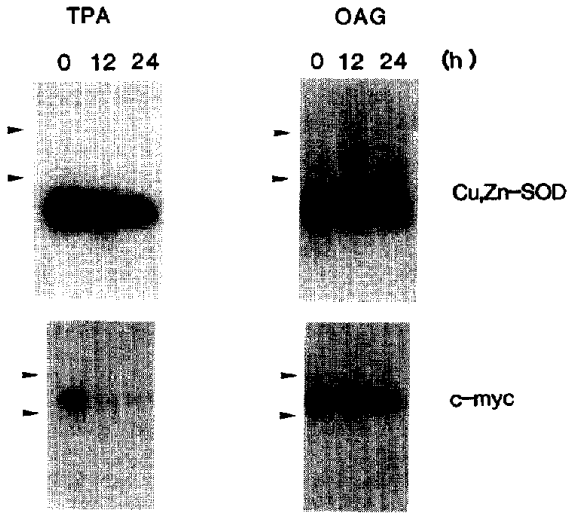

Fig.2. Effect of OAG on the level of CuZn-SOD mRNA in U937 cells. Cells were incubated with 50 ng/ml of TPA or 50 μg OAG and processed as in fig.1.

not undergo a detectable change in contrast to TPA-treated cells. To see whether the decrease in CuZn-SOD mRNA in differentiated U937 cells is unique to this particular cell line, its level in promyelotic leukemia HL-60 cells was examined. HL-60 cells differentiate into macrophage-like cells when treated with TPA and into granulocyte-like cells with DMSO. Levels of CuZn-SOD mRNA and c-*myc* were found to decrease by similar kinetics in cells treated with either TPA or DMSO (fig.3).

TPA treatment of B cell leukemia Raji cells or normal human diploid fibroblast JHU-1 cells failed to give rise to a detectable decrease in SOD mRNA (data not shown).

In table 1, the changes in the activity of CuZn-SOD in crude extracts of U937 cells following treatment with TPA are shown. The activity decreased after TPA treatment, but there was a lag period of about 12 h.

In the present communication, evidence is presented of the decrease in CuZn-SOD mRNA in U937 and HL-60 cells treated with TPA or DMSO during differentiation. mRNA for extracellular SOD could not be detected in U937 or HL-60 cells by the synthetic oligonucleotide probe described in section 2, even though it was found in human fibroblasts (data not shown).

SOD may perform some essential role in the

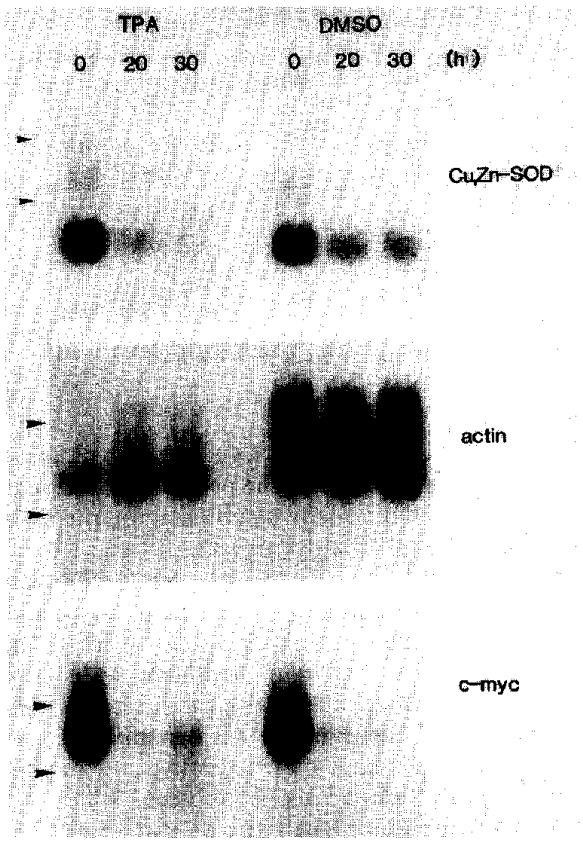

Fig.3. Changes in the level of CuZn-SOD mRNA during differentiation of HL-60 cells. HL-60 cells were treated either with 50 ng/ml TPA or 1.5% DMSO for the times indicated, and total RNA was analyzed as described in the legends for fig.1.

metabolism of active oxygens [16,25]. The activity of CuZn-SOD is known to change during the process of differentiation. Recent findings indicate that this activity increases during differentiation of mouse erythroid leukemia cells [26] or during muscle and adipocyte differentiation of rat embryo cells [27]. CuZn-SOD activity is also known to increase during lung development [17] or oxidant stress [28]. On the other hand, myeloperoxidase activity is reported to be less in mature granulocytes than in immature HL-60 cells [29].

Differentiated U937 or HL-60 cells produce active oxygens [9,30], and the authors initially expected that CuZn-SOD mRNA would increase during differentiation as a protective measure against the cytotoxic effects of active oxygens. The

Table 1

Change in CuZn-SOD activity during differentiation of U937 cells

Time of TPA-treatment (h)	CuZn-SOD activity (U/mg protein)
0	1.09 ± 0.12[a]
6	1.18 ± 0.10
12	1.22 ± 0.13
24	0.69 ± 0.24
48	0.34 ± 0.12

[a] ±, standard deviation from triplicate assays

decrease in CuZn-SOD mRNA followed by that of its activity, as observed in the present communication, may be biologically significant for the efficient production of active oxygens.

Cancer cells generally possess less SOD than their normal counterparts [31], and SOD and catalase activities are reported to decrease in mouse epidermis following treatment with TPA [32]. These findings along with the present results indicate that the level of SOD is dynamically controlled, and its change may be biologically important in utilization of active oxygens, which we consider important signals for cellular growth as well as for differentiation.

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